novel natural products by genome mining without requiring genetic manipulation of the producing organism, linkage between the metabolite isolated and the orphan gene cluster hypothesized to direct its production still needs to be established. This is usually achieved by demonstrating that inactivation of a biosynthetic gene abrogates production of the metabolite or that transfer of the gene cluster to a heterologous host results in metabolite production (Figure 1). Thus, genetic manipulations are ultimately still required.

It will be interesting to see whether the genomisotopic approach can be applied to the identification of other novel compounds whose existence is implicated by analysis of genome sequence data. Continued development of predictive bioinformatics tools for natural product biosynthetic machinery is essential to ensure broad applicability of the genomisotopic approach. Nevertheless, the recent emergence of diverse methodologies for new natural product discovery by genome mining may provide the impetus needed to nudge pharmaceutical companies back into large-scale natural product drug discovery programs.

REFERENCES

1. Gross, H., Stockwell, V.O., Henkels, M.D., Nowak-Thompson, B., Loper, J.E., and Gerwick, W.H. (2007). Chem. Biol. *14*, this issue, 53–63.

2. Koehn, F.E., and Carter, G.T. (2005). Nat. Rev. Drug Discov. *4*, 206–220.

3. Bentley, S.D., Chater, K.F., Cerdeno-Tarraga, A.M., Challis, G.L., Thomson, N.R., James, K.D., Harris, D.E., Quail, M.A., Kieser, H., Harper, D., et al. (2002). Nature *417*, 141–147.

4. Omura, S., Ikeda, H., Ishikawa, J., Hanamoto, A., Takahashi, C., Shinose, M., Takahashi, Y., Horikawa, H., Nakazawa, H., Osonoe, T., et al. (2001). Proc. Natl. Acad. Sci. USA *98*, 12215–12220.

5. Paulsen, I.T., Press, C.M., Ravel, J., Kobayashi, D.Y., Myers, G.S., Mavrodi, D.V., DeBoy, R.T., Seshadri, R., Ren, Q., Madupu, R., et al. (2005). Nat. Biotechnol. *23*, 873–878.

6. Bok, J.W., Hoffmeister, D., Maggio-Hall, L.A., Renato, M., Glasner, J.D., and Keller, N.P. (2006). Chem. Biol. *13*, 31–37.

7. Fazio, G.C., Xu, R., and Matsuda, S.P.T. (2004). J. Am. Chem. Soc. *126*, 5678–5679.

8. Lautru, S., Deeth, R.J., Bailey, L.M., and Challis, G.L. (2005). Nat. Chem. Biol. *1*, 265–269.

9. Challis, G.L., and Ravel, J. (2000). FEMS Microbiol. Lett. *187*, 111–114.

10. Tohyama, S., Eguchi, T., Dhakal, R.P., Akashi, T., Otsuka, M., and Kakinuma, K. (2004). Tetrahedron *60*, 3999–4005.

11. McAlpine, J.B., Bachmann, B.O., Piraee, M., Tremblay, S., Alarco, A.-M., Zazopoulos, E., and Farnet, C.M. (2005). J. Nat. Prod. *68*, 493–496.

12. Banskota, A.H., McAlpine, J.B., Sorensen, D., Aouidate, M., Piraee, M., Alarco, A.-M., Omura, S., Shiomi, K., Farnet, C.M., and Zazopoulos, E. (2006). J. Antibiot. (Tokyo) *59*, 168–176.

13. Banskota, A.H., McAlpine, J.B., Sorensen, D., Ibrahim, A., Aouidate, M., Piraee, M., Alarco, A.-M., Farnet, C.M., and Zazopoulos, E. (2006). J. Antibiot. (Tokyo) *59*, 533–542.

14. Xiong, Q., Wilson, W.K., and Matsuda, S.P.T. (2006). Angew. Chem. Int. Ed. Engl. *45*, 1285–1288.

15. Lin, X., Hopson, R., and Cane, D.E. (2006). J. Am. Chem. Soc. *128*, 6022–6023.

16. Scherlach, K., and Hertweck, C. (2006). Org. Biomol. Chem. *4*, 3517–3520.

17. Fischbach, M.A., and Walsh, C.T. (2006). Chem. Rev. *106*, 3468–3496.

18. Wenzel, S.C., Meiser, P., Binz, T.M., Mahmud, T., and Müller, R. (2006). Angew. Chem. Int. Ed. Engl. *45*, 2296–2301.

19. Song, L., Barona-Gomez, F., Corre, C., Xiang, L., Udwary, D.W., Austin, M.B., Noel, J.P., Moore, B.S., and Challis, G.L. (2006). J. Am. Chem. Soc. *128*, 14754–14755.

20. Wietzorrek, A., and Bibb, M.J. (1997). Mol. Microbiol. *25*, 1181–1184.

A Designed RNA Shuts Down Transcription

Julia E. Weigand[1] **and Beatrix Suess**[1,*]
[1] Lehrstuhl für Mikrobiologie, Friedrich-Alexander-Universität Erlangen-Nürnberg, Staudtstr. 5, 91058 Erlangen, Germany
*Correspondence: bsuess@biologie.uni-erlangen.de
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Using an in vivo selection strategy, Kehayova and Liu identified an RNA molecule capable of silencing gene transcription in yeast [1]. In doing so, they expand the repertoire of RNA molecules performing long-believed "protein-only" cellular functions.

The discovery of catalytic RNA more than 20 years ago initiated a new era of RNA research [2, 3]. This first discovery that RNA can be both an informational molecule and a catalyst caused a shift in thinking about RNA in general. In a virtually continual expansion, a plethora of RNA molecules with diverse catalytic and regulatory functions have emerged, indicating that RNA molecules can perform crucial roles within the cell once believed to be unique to the realm of proteins. The molecular basis for this diversity originates from the conformational flexibility and functional versatility of this macromolecule. In many ways, RNA seems to be more akin to proteins than to the chemically related DNA. Like proteins, RNA can adopt complex three-dimensional structures for the precise presentation of chemical moieties that are essential for its function as a biological catalyst, structural scaffold, or regulator of gene expression.

Molecular engineers began to harness Darwinian evolution in combination with rational design to optimize the known functions of RNA, but also to create "new" ones. For example,

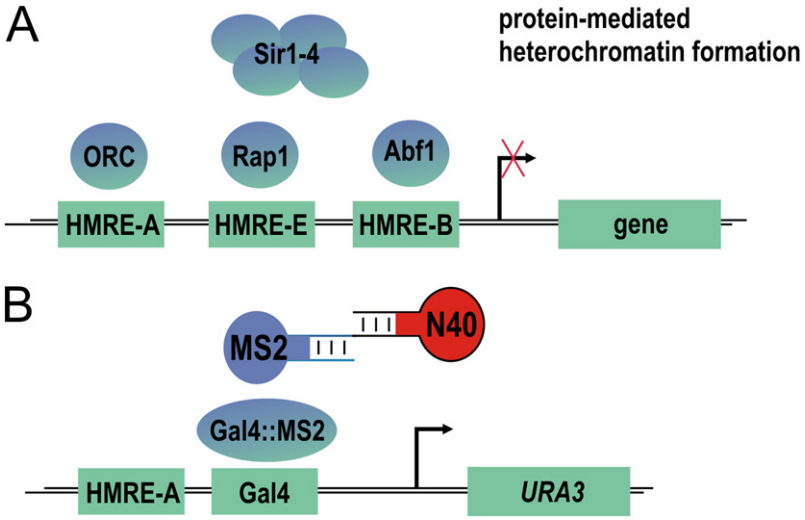

Figure 1. Selection Scheme for an RNA-Based Transcriptional Silencer

(A) Transcriptional silencing at the HMR-E locus. Binding of regulatory proteins ORC, Rap1, and Abf1 leads to recruitment of the Sir proteins and establishes a heterochromatic state that results in gene silencing.

(B) An MS2 hairpin-linked N_{40}-RNA library transcribed from a PolIII promoter localizes to the promoter of an *URA3* reporter gene through the respective DNA and RNA binding activities of a MS2 coat protein-Gal4 DNA binding domain fusion protein (Gal4::MS2). RNAs capable of silencing the expression of the *URA3* reporter gene enable survival on media containing 5-FOA.

RNA-based molecular switches and sensors were engineered [4, 5] and developed for conditional control of gene expression [6]. The subsequent discovery of natural riboswitches revealed that this principal was actually perfected millions of years ago by nature [7]. The engineered RNA-based regulators make use of a variety of mechanisms to exert their control. In bacteria, they target mainly translation initiation by inhibiting access to the ribosomal binding site [8, 9]. Eukaryotic switches target ribosomal access by preventing cap binding [10], but can also interfere with steps of gene expression further downstream like scanning, splicing, and mRNA degradation (reviewed in [11]). Moving away from negative regulation, Liu and colleagues evolved an RNA-based transcriptional activator capable of activating gene expression in yeast, probably by recruiting components of the transcriptional machinery [12].

In this issue of *Chemistry & Biology*, Kehayova and Liu expand the scope of designer RNAs by selection of an RNAi-independent transcriptional silencer [1]. Their evolution strategy was based on transcriptional silencing at the HMR-E locus (Figure 1A). The HMR-E silencer consists of binding sites for three essential factors: the origin recognition complex (ORC) and the two transcription activators Rap1 and Abf1. These factors recruit, via protein-protein interactions, the silent information regulator proteins Sir1-4, leading to heterochromatin formation and subsequent silencing of downstream genes [13]. Using a yeast three-hybrid system, the authors evolved an RNA molecule to replace the recruiting proteins (Figure 1B). In the selection strain used, the E and B sites of the HMR-E locus were replaced with a Gal4 binding site. This alteration results in the loss of gene silencing and the expression of a *URA3* reporter gene. An RNA-library, consisting of an N_{40} variable region sandwiched between MS2 RNA hairpins and a RNA polymerase III RNase P RNA (RPR) terminator, was then localized to the *URA3* promoter region through the DNA- and RNA-binding activities of an MS2 coat protein-Gal4 DNA binding domain fusion protein. To identify active RNAs, they performed classical FOA selection: RNAs capable of silencing the expression of the *URA3* gene enable survival on media containing 5-fluoroorotic acid (5-FOA), which otherwise would lead to rapid cell death by URA3-mediated conversion of the compound into the toxic 5′ fluorouridine monophosphate.

After two rounds of selection, clones capable of growing in the presence of 5-FOA were identified. One of the clones isolated showed activity comparable to known transcriptional repressors directly recruited to the HMR-E locus by fusion to the Gal4 binding domain [14]. Random mutations were introduced into this silencing-active clone, and the resulting RNA library subjected to an additional round of selection; this led to the isolation of an even more potent silencer than the parental RNA. Furthermore, a detailed mutagenesis study was performed to elucidate the structural properties of the most active RNA-transcriptional repressor. Finally, using genetic studies, the authors provided evidence that the evolved RNA establishes gene silencing via the traditional Sir protein-dependent mechanism. Deletion of any of the four Sir proteins required for HMR-E repression abolished silencing through the RNA completely. Furthermore, they demonstrated that deletion of all three silencer (HMR-E A, E and B) elements allows only a low level of repression. This result is consistent with previous findings that full silencing of the HMR-E locus depends on a least two active silencer elements [15].

There are at least three remarkable aspects of this report: (1) the identification of a potent silencer from a modest library size, (2) the ability of RNA to silence a gene independent of the RNAi pathway, and (3) the ability to select an RNA molecule with a desired in vivo function. It is surprising that it was possible to identify a potent silencer from an RNA library of only 5×10^4 members. This may be explained by the fact that the target was not a single component like a regulatory protein. Instead, an entire biological process was targeted which increases the number of potential interaction partners. Thereby, the success rate of directed evolution is advanced and allows a dramatic reduction in the required complexity of the initial selection pool. This approach clearly shows the tremendous potential of in vivo

RNA selection. It is also important to highlight that the evolved RNA silences gene expression independent of the RNAi pathway. This observation suggests that, although there are no known RNA-based transcriptional silencers, and *S. cerevisiae* seems to lack proteins related to the RNAi pathway [16], natural RNA-based gene silencing may also exist in budding yeast. By using functional versatility combined with the powerful ways in which RNA can be manipulated and characterized, Kehayova and Liu nicely demonstrate that an in vivo active RNA molecule with a desired intracellular function can be developed. Thereby, they extend the scope of in vivo functional RNAs that can be used as regulatory elements. Furthermore, their straightforward design and use of a simple selection system promises to add more custom-crafted RNA molecules as novel tools to the experimental repertoire of molecular biology.

REFERENCES

1. Kehayova, P.D., and Liu, D.R. (2007). Chem. Biol. *14*, this issue, 65–74.
2. Kruger, K., Grabowski, P.J., Zaug, A.J., Sands, J., Gottschling, D.E., and Cech, T.R. (1982). Cell *31*, 147–157.
3. Guerrier-Takada, C., Gardiner, K., Marsh, T., Pace, N., and Altman, S. (1983). Cell *35*, 849–857.
4. Breaker, R.R. (2002). Curr. Opin. Biotechnol. *13*, 31–39.
5. Silverman, S.K. (2003). RNA *9*, 377–383.
6. Werstuck, G., and Green, M.R. (1998). Science *282*, 296–298.
7. Winkler, W., Nahvi, A., and Breaker, R.R. (2002). Nature *419*, 952–956.
8. Desai, S., and Gallivan, J. (2004). J. Am. Chem. Soc. *126*, 13247–13254.
9. Suess, B., Fink, B., Berens, C., Stentz, R., and Hillen, W. (2004). Nucleic Acids Res. *32*, 1610–1614.
10. Hanson, S., Berthelot, K., Fink, B., McCarthy, J.E., and Suess, B. (2003). Mol. Microbiol. *49*, 1627–1637.
11. Bauer, G., and Suess, B. (2006). J. Biotechnol. *124*, 4–11.
12. Buskirk, A.R., Kehayova, P.D., Landrigan, A., and Liu, D.R. (2003). Chem. Biol. *10*, 533–540.
13. Rusche, L.N., Kirchmaier, A.L., and Rine, J. (2003). Annu. Rev. Biochem. *72*, 481–516.
14. Chien, C.T., Buck, S., Sternglanz, R., and Shore, D. (1993). Cell *75*, 531–541.
15. Andrulis, E.D., Zappulla, D.C., Alexieva-Botcheva, K., Evangelista, C., and Sternglanz, R. (2004). Genetics *166*, 631–635.
16. Nakayashiki, H. (2005). FEBS Lett. *579*, 5950–5957.